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29724

ABSTRACT

The Pseudomonas, Flavobacterium and Achromobacter group of bacteria and some micrococci are capable of developing at low temperature: Bact.putidum and Achromobacter sp. develop at -2°; Bact. fluorescens, Flavobacterium flavescens, Flavobacterium ochraceum, Flavobacterium sp., and Micrococcus sp. increase at -5°, and Flavobacterium sulfureum and Bact. lactis viscosum can multiply even at -8°. The other groups of bacteria, including those of the paratyph~~ees~~oid group and B. botulinus, do not develop at a temperature above +2°. Thus the minimum temperature required for bacterial development is determined by the group to which the particular bacteria belong. The lowest temperature at which yeast can develop is about -8°. The microorganisms not resistant to cold, gradually die off at low temperature, in some cases rather slowly. The spore-forming types of bacteria are more resistant to cold. The addition of salt to the nutritive media lowers the bacteria's resistance to cold. AUTHOR /577*

*Numbers given in margin indicate pagination in original foreign text.

Claims that certain species of bacteria and types of yeast can develop at temperatures considerably below zero have recently been appearing in literature with increasing frequency. /565

Such an opinion was expressed by Stewart (1934), for example, in his study of the preservation of fish. He says that at -6° the possibility of the development of bacteria is not excluded, and that -12° is the lowest possible temperature for such development. Berry (1937) arrives at similar conclusions in a number of studies. He believes that the "deadline" lies somewhere between -6.6° and -9.4°. In a study he made jointly with Magoon (1934), he observed the development of microorganisms at -4° and -6.7°. Ten of the 71 cultures of microorganisms isolated by Bedford from sea water (as quoted from Stewart) were found capable of growing at -7.5°, and 12 cultures could develop at a minimum temperature of -5°. The growth of the microorganisms at -10° could not be observed. General references to the capacity of marine bacteria to grow at low temperatures are found in a number of studies by Hess (1934).

Smart (1935) reports that some of the microorganism cultures she isolated from frozen products continued to grow at -8.89°. There were eight such cultures: three of them belonged to the Saccharomyces yeast group, four were Bac. aterrimus L and N, B. fluorescens Ford., B. mycoides Fl., and B. ruminatus Got, and one was a mold.

Haines (1937) believes that -10° is the lowest possible temperature at which microorganisms can grow. He divides all the microorganisms into three groups in relation to temperature. The first group consists of staphylococci and many pathogenic bacteria. The optimum temperature for their development is 37°. The minimum temperature is $+10^{\circ}$. They do not develop at temperatures below $+10^{\circ}$. The next group includes bacteria of the Coli and Proteus type, whose growth stops at about +5°, and, finally, the third and most interesting group consists of Achromobacter and Pseudomonas type of bacteria and yeasts. This group of microorganisms is capable of developing at temperatures between -3 and -5°.

Our investigation into the microflora of frozen berries (Chistyakov and Noskova (1935)) showed that berries kept in a chamber at about -5° revealed an increase in the number of microorganisms which ceased to grow and died off at -8°. /566

The purpose of this study was to establish the temperature range favorable to the development of some of the types of bacteria and yeast found in food products.

Methods

The experiment was a follow-up of our previous study of the preservation of frozen berries, and involved the use of pure cultures in laboratory media. We had established at that time that the storaging of berries at -5-6° increases the number of microorganisms. The question we sought an answer to was what group of organisms accounted for that increase, and at what temperature does a particular group of microorganisms cease to grow. Our efforts to ascertain the limiting temperatures in natural media failed to produce any definite results because of certain imponderables.

Forty-three different bacterial and yeast cultures were examined in the experiment. These included: 7 strains of the coli-paratyphoid group--Bact. coli commune Esch., Bact. paratyphi A, Bact. paratyphi B, Bact. coli var. albidoliquefaciens Lehm. and Levy; two Aerobacter aerogenes Beijerink strains; one Proteus vulgaris Hauser strain; two species of lactate bacteria--Bact.

prodigiosum L and N. and Bact. cartovorum Lehm.; one species of each Sarcina and micrococci; three species of the Subtilis-Mesentericus group; two B. megatherium strains; two species of the Pseudomonas family (Bact. fluorescens L. and N. and Bact. putidum L. and N.); seven species of the Flavobacterium family; two Achromobacter species; three spore-forming anaerobes--B. botulinus B., B. sporogenes and B. amylobacter, and, finally, ten different yeast cultures. A large number of the above-listed bacteria and yeast were isolated directly from the food products. The paratyphoid bacteria and all the three anaerobic species were taken from a laboratory collection.

Institute's Cold Chambers at the Following Temperatures:
-12°, -8°, -5, -2°, $\pm 0^{\circ}$ and $+2^{\circ}$

Ordinary peptone agar in test tubes was used as a solid medium. Following Prof. Horowitz-Vlasova's experiment in her study titled "The Problem of Psychrophilic Microbes" (1933), we used agar with 3 and 6 percent NaCl in addition to the usual media; it turned out, however, that these media were not in any way superior to the normal ones. At -8° we also used peptone gelatin with 20 percent sugar as a medium. In the case of yeast we used ~~an oblique~~ a wort agar slant with a varying sugar content. The anaerobic cultures were made in a so-called semi-liquid agar (0.5 percent agar instead of the usual 1.5 percent).

The inoculation on solid media was made by the use of a platinum loop to cover them with a suspension of microorganisms which had been washed off the agar culture. Liquid media were inoculated with one- or two-day bouillon cultures.

The behavior of the microorganisms in liquid media was observed by way of periodic inspections, during which the time of the incipient growth and degree of development was noted. The development in liquid media was determined by counting the colonies on dishes.

The duration of the observation of various cultures on solid media ranged from 9 to 14 months, and in liquid media from 1 to 5 months.

The Experiment

Twelve of the 30 examined strains of aerobic bacteria were found to be capable of growth at temperatures below zero. These bacteria are listed in table 1.

TABLE 1. DEVELOPMENT OF BACTERIA AT LOW TEMPERATURES ON SOLID MEDIA.

Organism	Test temperatures and duration of development until well pronounced growths (in days)				
	−8°	−5°	−2°	±0°	+2
Bact. putidum	No growth after 9 months	No growth after 9 months	18	12	8
Achromobacter sp.	The same	The same	18	12	7
Bact. fluorescens	"	15	12	9	6
Micrococcus sp.	"	15—20	7—10	5—8	2—6
Flavobacterium ochraceum, strain 1	"	15—20	7—10	5—8	2—6
Flavobacterium ochraceum, strain 2	"	15—20	7—10	5—8	2—6
Flavobacterium lutescens Ber.	"	15—20	7—10	5—8	2—6
Flavobacterium sp., strain 1	"	15—20	7—10	5—8	2—6
Flavobacterium sp., strain 2	"	15—20	7—10	5—8	2—6
Flavobacterium sulfureum, strain 1	206	15—20	7—10	5—8	2—6
Flavobacterium sulfurum, strain 2	206	15—20	7—10	5—8	2—6
Bact. lactis viscosum Lehm and Neum.	206	15—20	7—10	5—8	2—6

As indicated in table 1, all of the listed 12 strains are capable of /567 developing fairly rapidly not only at 0° but also at -2°; ten of them begin to grow at -5°, and three at -8°.

The capacity of these bacteria to develop at low temperatures has frequently been noted in literature. Berry and Magoon (1934), for example, report that in their experiments Bact. fluorescens liquefaciens developed at a temperature of -4°. The same bacteria were found to grow at -8.89° in Smart's experiments (1937). The psychrophilic characteristics of the Flavobacterium group were noted by Stewart (1934), Smart and Brunstetter (1934, 1937), etc.

Tests were made in liquid cultures and a quantitative account kept of the developing bacteria with a view to a fuller description of the rate of their development at freezing temperatures.

The data on those tests are cited in tables 2, 3, 4, and 5 and in figure 1.

The following four species of bacteria were found to be less viable at low temperatures: Bact. prodigiosum, Bact. carotovorum, Bact. coli var. and Sarcina sp.; the minimum temperature required for their development is apparently

TABLE 2. RATE OF DEVELOPMENT OF BACT. FLUORESCENS IN PEPTONIZED MEAT BOUILLON AT LOW TEMPERATURES.

Initial number of bacteria in 1 cm³ — 21,200,000

Temperature of experiment	Duration of development (in days): 1	3	8	15	21	24	35
	Number of bacteria in 1 cm³ (in thousands)						
+20°	390 000	1 800 000					
+2°		39 000	473 000	1 570 000			
±0°		24 000	67 000	1 430 000			
−2°		14 000	28 700	900 000	980 000		
−5°		11 400	24 000	64 400	80 000	91 700	275 000

TABLE 3. RATE OF BACT. PUTIDUM DEVELOPMENT IN PEPTONIZED MEAT BOUILLON AT LOW TEMPERATURES .

Temperature of experiment	Duration of development (in days)					
	1	3	8	15	21	35
	Number of bacteria in 1 cm^3 (in thousands)					
+20°	213 000	2 550 000				
+2°		41 200	147 000	851 000		
±0°		38 000	51 400	295 000	850 000	
−2°		33 000	33 200	105 600	225 000	
−5°		30 500	27 600	26 000	28 000	28 000

TABLE 4. RATE OF BACT. LACTIS VISCOSUM DEVELOPMENT IN PEPTONIZED MEAT BOUILLON AT LOW TEMPERATURES.

Temperature of experiment	Duration of development (in days)		
	4	8	24
	Number of bacteria in 1 cm^3 (in thousands)		
+20°	406 000		
+2°	63 500	272 000	
±0°	25 500	125 000	
−2°	23 500	122 000	290 000
−5°	1 890	7 650	92 000

TABLE 5. RATE OF ACHROMOBACTER SP. DEVELOPMENT IN PEPTONIZED MEAT BOUILLON AT LOW TEMPERATURES.

Temperature of experiment	Duration of development (in days)			
	2	6	10	26
	Number of bacteria in 1 cm^3 (in thousands)			
+20°	94 200	340 000		
+2°	11 100	121 400	129 000	
±0°	4 300	66 000	87 500	
−2°	2 120	29 400	82 100	97 000
−5°				520

about 0°. Thus in the case of Sarcina sp. the first signs of growth in a solid medium at +2° in the form of scattered very tiny colonies were observed on the 15th day, at 0° on the 22nd day and at -2° only on the 87th day. The development did not proceed beyond those small colonies. /569

In the case of Bact. coli var. albidoliquefaciens, signs of growth at +2° were detected on the 17th day, and at 0° on the 176th day, but no well pronounced growth could be observed in the following 14 months.

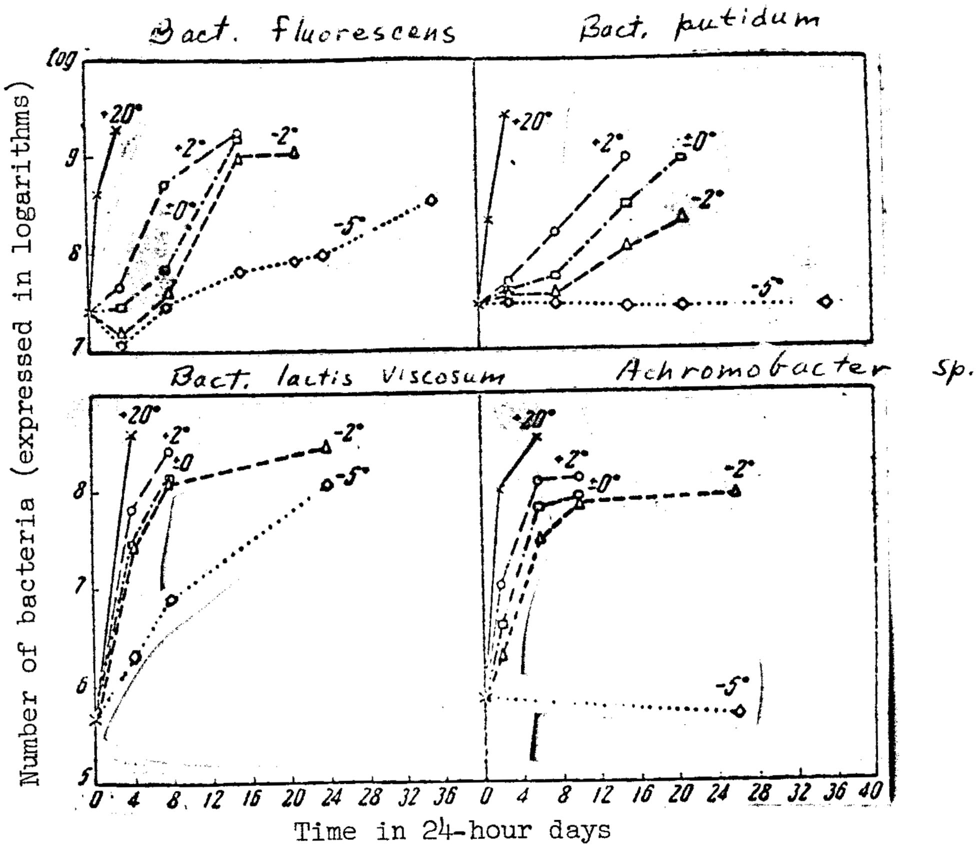

Figure 1

At a temperature of +2° the Bact. prodigiosum began to grow 73 days after inoculation, and Bact. carotovorum, under similar conditions, only after 149 days. These bacteria were not observed to grow at 0° or -2° over a period of 14 months.

Experiments with the liquid cultures of these bacteria also revealed that they are limited to the +2 to -2° temperature range. At these tempera- /572 tures the bacteria failed to develop and died off gradually, as may be judged from the tables 6, 7 and 8 and figure 2 cited below.

Finally, the last group of bacteria consisting of the following species and strains failed to grow at the temperatures used: Bact. coli commune, Bact. paratyphi A., Bact. paratyphi B., two strains of Bact. megatherium and B.

TABLE 6. TESTING BACT. COLI VAR. ALBIDOLIQUEFACIENS IN PEPTONIZED MEAT BOUILLON AT LOW TEMPERATURES.

Temperature of experiment	Initial number of bacteria	Duration of testing (in days)						
		2	5	12	61	130	138	157
	Number of bacteria in 1 cm³ (in thousands)							
+20°	15 500	5 060 000	5 550 000	1 640 000				
+2°	14 450	13 900	11 800	19 825	5 690 *			
±0°	14 400	14 300	9 300	6 800	2 100	510	298	
−2°	14 [illegible]00	13 700	11 800	11 850	4 130	1 000		680
−5°	16 500		12 000	13 740	455	31		3

TABLE 7. TESTING BACT. COLI VAR. ALBIDOLIQUEFACIENS IN PEPTONIZED MEAT BOUILLON WITH 6 p.c. NaCl AT LOW TEMPERATURES.

Temperature of experiment	Initial number of bacteria	Duration of testing (in days)			
		2	5	57	134
	Number of bacteria in 1 cm³ (in thousands)				
+20°	4 000	630 000	166 400		
+0°	8 400	6 780	8 700	94	9,7
±2°	8 900	5 870	3 200	26	4,5
−5°	7 450	5 900	4 800	10	1,5
−8°	8 100	7 300	6 420	2,7	0

Ellenbachensis and the anaerobic bacteria B. botulinus B., B. sporogenes and B. amylobacter; consequently the temperature favorable to the development of these bacteria is somewhere above $+2^{o}$.

This has been supported also by the experiments made with three of these cultures in a liquid medium (tables 9-12, and figure 3).

All but one of the tested 10 types of yeast were found to be capable of growth at -5^{o}, and one of them developed at a temperature of -8^{o} (table 13).

Bact coli var albidolique faciens

in bouillon without salt

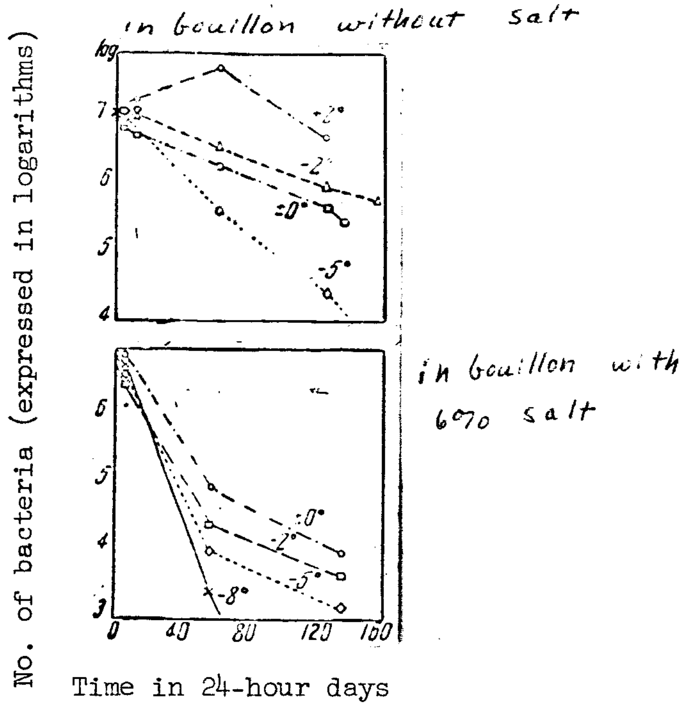

Figure 2

in bouillon without salt in bouillon with 3% salt

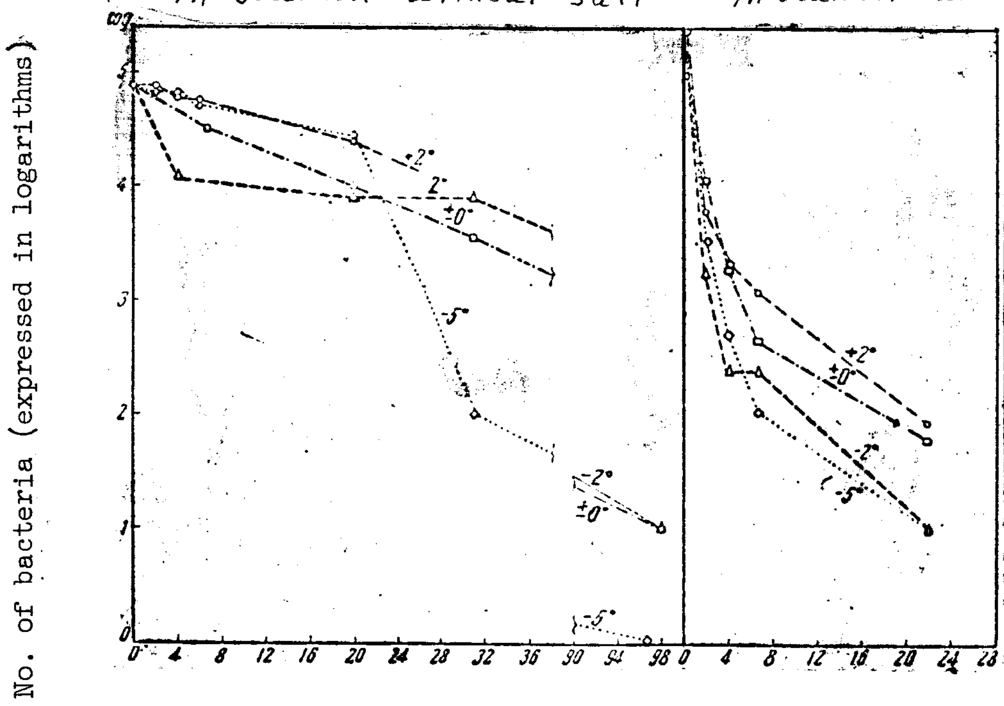

Figure 3

TABLE 8. TESTING BACT. PRODIGIOSUM IN PEPTONIZED MEAT BOUILLON AT LOW TEMPERATURES.

Temperature of experiment	Duration of testing (in days): 4	12	18	[illegible]
	Number of bacteria in 1 cm³ (in thousands)			
+20°	2 050 000			
+2°	25 300	5 600	3 000	2 840
±0°	26 700	17 000	13 500	11 700
−2°	26 000	20 000		960
−5°	23 300	22 400	15 400	14 500

TABLE 9. TESTING BAC. ELLENBACHENSIS IN PEPTONIZED MEAT BOUILLON AT LOW TEMPERATURES.

Temperature of experiment	Initial number of bacteria	Duration of testing (in days): 2	6	46	[illegible]	105
		Number of bacteria in 1 cm³ (in thousands)				
+20°	1920	61 000	12 000			
+2°	2680	1 040	155	Grew over with moulds		
±0°	4320	540	114	70		
−2°	1960	200	43	Not more than 100 cells	Not more than 10 cells	
−5°	2020	130	26	Not more than 100 cells	Not more than 10 cells	

Two of the 13 species of yeast listed in table 13, Apiculatus and Pichia sp., grew considerably more slowly than the others at temperatures of -2^{o} and -5^{o}, and did not achieve the full growth, achievable at room temperature, over a period of 14 months.

All these 10 species of yeast were isolated from frozen or chilled products. Apiculatus, Torula rubefaciens, pink yeast, Pichia sp. and yeast No. 6 /574

TABLE 10. TESTING LACTOBACILLUS SP. IN PEPTONIZED MEAT BOUILLON WITH AN ADDITION OF 3 PERCENT SALT AT LOW TEMPERATURES.

Temperature of experiment	Initial number of bacteria	Duration of testing (in days) 2	5	45	113
	Number of bacteria in 1 cm^3 (in thousands)				
+20°	2760	13 000	29 500	12 100	
+ 2°	3750	2 740	3 130	1 300	
± 0°	3250	750	124		40
− 2°	3150	3 750	2 440	800	
− 5°	3000	2 320	2 750	549	6

TABLE 11. TESTING AEROBACTER AEROGENES IN PEPTONIZED MEAT BOUILLON AT LOW TEMPERATURES.

Temperature of experiment	Duration of testing (in days) 1	2	4	7	20	31	98
	Количество бактерий в 1 cm^3 (в тыс.) Number of bacteria in 1 cm^3 (in thousands)						
+20°	480 000 000	1 000 000 000	2 900 000 000	750 000 000	158 000 000	38 750 000	
+10°	8 350	57 500	46 750	38 950	1 000 000	70 000 000	
+ 5°	85 800	80 800	50 500	47 750	3 000	100	
+ 2°			62 250	56 400	20 700	10 200	
± 0°			55 900	36 500	8 650	5 786	Less than 10
− 2°			15 200		7 600	7 515	
− 5°			65 000	51 600	22 200	90	

and 7 (uncertain) were isolated from frozen berries, Torula (pink) from bouillon kept at -5°, Torula sp. from mutton, and another strain of Torula sp. from beluga fish kept in a refrigerator. Yeast No. 10 was isolated from a chilled soy sauce.

In addition to the species indicated in table 13, we tested 7 types of yeast that had been kept for a number of years in a laboratory collection.

TABLE 12. TESTING AEROBACTER AEROGENES IN PEPTONIZED MEAT BOUILLON WITH THE ADDITION OF 3 PERCENT NaCl AT LOW TEMPERATURES.

Temperature of experiment	Initial number of bacteria	Duration of testing (in days)				
		2	4	17	22	176
		Number of bacteria in 1 cm^3 (in thousands)				
+20°	260 000	466 400 000	490 000 000	440 000 000	80	
+2°	250 000	7 700	2 190	1 210	60	
±0°	112 000	12 500	2 175	420		
−2°	202 500	2 050	250	250	Not more than 10	0
−5°	159 000	3 650	570	120	Not more than 10	0

TABLE 13. DEVELOPMENT OF YEASTS AT LOW TEMPERATURES ON SOLID MEDIA.

	Temperature of test and duration of development (in days) until clearly expressed growth				
	−8°	−5°	−2°	±0°	+2°
Uncertain, No 6	206	15	8	6	4
Uncertain, No 7	No growth after 9 months	15	8	6	4
Pink yeast	No growth after 14 months	15	8	6	4
Torula sp.	No growth after 9 months	38	21	15	7
Torula sp.	Ditto	38	21	10	7
Torula (розовые) pink	Ditto	45	37	30	21
Torula rubefaciens	No growth after 14 months	197	80	50	25
Apiculatus	Ditto	197*	197*	165	165
Pichia sp.	Ditto	197*	166*	80	23
Uncertain, No 10	Ditto	No growth after 9 months			

The tests were made over a period of nine months at -2° temperature. Here are the results:

1. Sacch. exiguus--no growth.
2. Logos yeast--no growth.
3. Shteinberg yeast--no growth.
4. Sacch. intermedius--very poor growth.
5. Sacch. Kefir.--very poor growth. /575
6. Sacch. turbidans--clearly pronounced growth.
7. Yeast isolated from oak tree mucilage -- clearly pronounced growth.

The capacity of yeast to grow at low temperatures is discussed by Berry and Magoon (1934), Smart (1935), etc.

As pointed out earlier, only one of the 10 species of yeast we had tested at temperatures below -5° was capable of growing. But in one of our experiments with mold fungi (Chistyakov and Bocharov[1]) there was a case in which the yeast isolated from frozen pears in a 50 percent sugar syrup was capable of growing also at a -8° temperature. And the rate of its development, depending on the temperature, was determined by the following periods of time: at +2° symptoms of growth were noted 6 days later, at 0° after eight days, at -2° also after eight days, at -5° after 10 days, and at -8° after 90 days.

In all cases when the bacteria were found to be incapable of growing in cold weather, they gradually died off at all temperatures, from +2° down.

The bacteria we tested may be roughly divided into two groups, according to their death rate: some of them survive the cold and remain viable for over 14 months, and others die off completely within the first six months.

The first group includes all the spore-forming aerobic and anaerobic bacteria, and some sporeless species, such as Bact. prodigiosum, Bact. coli var., Sarcina sp., etc. (table 14).

The effect of low temperatures on the development of mold fungi, Microbiology, VII, 4th edition, 1938.

TABLE 14. DEVELOPMENT OF BACTERIA AT ROOM TEMPERATURE AFTER THE EFFECT OF LOW TEMPERATURE.

Culture	Duration of storing in cold (in months)	Storage temperature									
		−8°		−5°		−2°		±0°		+2°	
		1	2	1	2	1	2	1	2	1	2
Bac. Ellenbachensis Stutzer	6	6	+	6	+	6	++	6	++	6	+++
	10	8	70	5	60	5	++	8	++	5	++
	14	[illegible]	80	2	60	2	60	5	50	7	++
Bac. mesentericus Lehm. & Neum.	6			4	++	4	+++	4	+++	4	+++
	10	8	70	5	50	5	80	5	130	5	100
	14	11	20	2	50	2	70	2	++	2	++
Bac. subtilis F. Cohn	6	4	+++	3	+++	3	+++	6	+	3	++
	10	5	+++	5	++	5	++	5	14	5	50
	14	5	+++	2	+++	2	70	2	25	2	80
Bac. megatherium de Bary	6	14	+++	6	+			6		6	—
	10	8	+++	8	6	8	8	8	6	8	8
	14	11	8	2	5	5	4	5	3	7	7
Bac. megatherium de Bary	6	6	+	8	+		—		—		—
	10	5	2		—		—		—		—
	14	2	+++		—		—		—	5	2
Bact. carotovorum Lehm.	6	6	+	6	+++	4	+++	4	+++	2	+++
	10	5	+++	5	20	5	16	10	10	2	++
	14	2	+++	2	1		—			7	5
Bact. coli var. albidoliquefaciens Lehm. & Levy	6	14	+	4	+++	2	+++	4	+++	2	+++
	10	8	++	8	2	5	++	8	50	2	+++
	14	5	+++		—	7	20		—	5	+++
Sarcina sp.	6	4	++	2	+			2	+++	2	+++
	10	5	+++	5	+++	5	+++	3	+++	3	+++
	14	5	+++	2	+++	2	+++	7	+++	5	+++
Bact. prodigiosum Lehm и Neum.	6	5	++	4	+++	4	+++	4	+++	2	+++
	10	5	++	10	1	5	++	5	+++	2	+++
	14	2	+++		—		—		—	2	+++
Bact. paratyphi B.	6		++		++	4	+++	4	+++	3	+++
	10		—	10	2	5	+++	5	5	5	++
	14		—		—	11	1	—	—	2	+++
Aerobacter aerogenes Beijerink	6	4	++	4	+++	4	+++	4	+++	4	+++
	10	5	+	5	++	5	++	5	50	5	50
	14		—	11	1		—		—	11	2
Aerobacter aerogenes Beijerink	6		—	11	+	2	+	11	+	2	+++
	10		—		—		—		—	5	30
	14		—		—		—		—		—
Bact. coli commune Esch.	6		—	6	+	8	+	8	+	6	+
	10		—		—		—		—	16	1
	14		—		—		—		—		—

TABLE 14 (Continued)

Culture	Duration of storing in cold (in months)	Storage temperature −8° 1	−8° 2	−5° 1	−5° 2	−2° 1	−2° 2	±0° 1	±0° 2	+2° 1	+2° 2
Proteus vulgaris Hauser	6	8	+++	6	+++	2	+	6	++	4	+++
	10		—		—		—		—	5	++
	14		—		—		—		—		
Lactobacillus sp.	6		—	5	+++	6	+++	8	+++	6	+++
	10		—	5	++	8	12	8	30		—
	14		—	7	5		—		—		—
Lactobacillus sp.	6	6	+	4	+++	6	—		++	4	+++
	10		—		—		—	6	—		—
	14		—		—		—		—		—
Bact. paratyphi A	6		—		—		—		—		—
	10		—		—		—		—		—
	14		—		—		—		—		—

Note: Shown in the first column (1) is the time from the moment of the transfer of the culture from low to room temperature until the appearance of the first symptoms of growth. In the second column (2) is the degree of development during 12 to 14 days by the following symbols:

+++ uninterrupted growth on the surface of the agar at the place the culture was applied

++ uninterrupted growth, but thin

\+ not continuous growth but the colonies cannot yet be counted

— Figure means number of colonies in the case when growth is not continuous, but scattered in the form of isolated colonies

The second group consists of Bact. coli communis, Bact. paratyphi A, certain lactate bacteria and Proteus vulgaris.

Cited in table 14 are the data on the development of bacteria on ~~oblique~~ slants agar at room temperature after having been kept for 6, 10 and 14 months at low temperatures. There are two columns for each temperature. The time elapsing between the change of the culture from a low to room temperature and the appearance of growth symptoms is indicated in column 1, and the extent of the growth in 12-14 days at room temperature in column 2.

We should point out that in many cases the death rate of the bacteria in low temperatures was so high that their eventual development at room temperature on agar appeared in the form of separate colonies.

Curiously, the condition of the medium in these experiments at -5° and -8° did not have any marked effect on the death of the bacteria. The psychrophilic species were preserved on both the frozen (at -8° and in some cases at -5°) and non-frozen agar.

The anaerobic bacteria are not included in table 14. The three anaerobic species we had tested remained viable after a 14-month exposure to all temperatures from + 2 to -8°, and after a 10-month exposure to a -12° temperature; they revealed a sizable growth when changed to room temperature. Such a high resistance to cold on the part of these bacteria is apparently due to their spores, as in the case of the spore-forming aerobes.

Simultaneous experiments on ordinary agar and agar containing 3 and 6 percent NaCl revealed that in the latter case the survivability of the bacteria at low temperatures was lower, and that only the spore-forming species survived a 6-month exposure to temperatures ranging from $+2^{\circ}$ to -12°.

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